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## Automatic Counting of *Aedes aegypti* Eggs in Images of Ovitraps

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### 1. Introduction

Dengue is a disease caused by a virus and transmitted by the *Aedes aegypti* mosquito. The *Aedes aegypti* appeared in Africa (probably in the northeast region) and it was spread there to Asia and Americas, mainly through the maritime traffic. In Brazil, it arrived in the 18th century with the boats that carried slaves, since the eggs of the mosquito can resist without contact with the water for up to one year.

*Aedes aegypti* is a very efficient disseminator of human pathogens as a result of evolutionary adaptations to frequent haematophagy as well as to the colonization of countless types of habitats, associated with environmental and cultural factors that favor the proliferation of this mosquito in urban ecosystems (Regis et al., 2008). In average, each *Aedes aegypti* lives around 30 days and the female puts between 130 and 200 eggs in each gonadotrophic cycle. It is able to lay their eggs repeatedly along its life, if copulate with the male at least once. The sperm is stored in its spermathecae (a reservoir present inside of its reproductive system). After the mosquito acquires the dengue virus, the female becomes a permanent vector of the disease and may even pass to his successors, who have already born infected.

The eggs are not put directly in the water; they are placed millimeters above the surface, in places such as: empty cans and bottles, tires, gutters, pots of plants or any other place that can store rain water. When it rains and the water level rises, coming into contact with the eggs, the eggs hatch in about 30 minutes. Within 8 days the mosquito can complete its life cycle from egg, to larvae, to pupae and to an adult flying mosquito.

These mosquitoes are responsible for one of the most difficult public health problems in tropical and semi-tropical world: the epidemic proliferation of dengue, a viral disease that, in its most dangerous form, dengue hemorrhagic fever, can even cause death of affected human beings (Perich et al., 2003). In the absence of an effective preventive vaccine, effective treatment or chemoprophylaxis etiologic, the only way to reduce the dengue proliferation is the reduction of the potential breeding containers. This means the involvement of vector control personnel, several public administration sectors, social organizations, productive

sectors and the general community that indirectly contribute to the increasing number of breeding containers (Perich et al., 2003) (Dibo, et al. 2005) (Regis et al. 2008). The early detection to outbreak diseases such as dengue is important to enable shares of research and monitoring by the agencies of public health, which reinforces the need for surveillance systems. The routinely employed method to monitor *Aedes aegypti* population in Vector Control Programs of Brazilian states is larval surveillance in potential breeding containers, which enables the attainment of entomological indicators such as the Premise, Container and Breteau Indexes (Dibo et al., 2005). In non-infected municipalities, this Program recommends the use of larvitrap, mat-black containers (in fact, sections of tires) containing 1 liter of water that are checked on a weekly basis, aiming at detecting foci of *Aedes aegypti* (Dibo et al., 2005).

One of the most efficient methods available for mosquito detection and monitoring is the use of ovitraps, which consist of black containers that are partially filled with tap water holding vertical wooden paddles with one rough side. Ovitrap are sensitive, fast, and economic to determine the presence of egg-laying females of *Aedes aegypti* (Dibo et al., 2005) (Gama, Eiras & Resende, 2007).

To generate important statistics and furnish government agencies and vector control programs information enough to project official actions and programs to develop and increase the control of dengue mosquitoes, it is very important to count the number of *Aedes aegypti* eggs present in ovitraps. This counting is usually performed in a manual, visual and non-automatic form. To aid the control of dengue proliferation, this work approaches the development of automatic methods to count the number of eggs in ovitraps images using image processing, particularly color segmentation and mathematical morphology-based non-linear filters.

In Recife, Brazil, the research on dengue is made mainly by the The Aggeu Magalhaes Research Center (CPqAM). This research is part of a project called SAPIO, granted by FINEP, that aims the development of new technologies for dengue control, surveillance and information dissemination.

This Chapter is organized as follows: next Section describes the images acquired and the algorithms developed to perform automatic counting of *Aedes aegypti* eggs in ovitraps. Following, the results are related and analyzed in Section III. In Section IV it is presented conclusions and performed some commentaries on our results.

## 2. Material and Methods

For this experiment, we used a digital camera with: 7.2 Megapixels resolution, LCD 2.5'', 4.5 times Optical Zoom and LEICA DC Vario Elmarit lens. The ovitrap was digitized with about 700 dpi resolution and 4 times optical zoom. This process generated a true color digital image of 3,072 *versus* 2,304 pixels which was split into sub-images for the experiments. The amount of eggs in each one of these sub-images is acquired by visual inspection allowing an easy comparison with our new proposal. Figure 1 presents some sample sub-images used in the tests and the amount of eggs in each one of them. The same figure also presents a zooming into some *Aedes aegypti* eggs. The images are digitized in RGB color system due to the camera features.

One of the problems of an automatic counting method is the segmentation of the images (Parker, 1997). A segmentation algorithm divides an image into its relevant objects. As the

concept of object can be different from image to image, segmentation is not a simple task. Classical segmentation algorithms can be found as watershed (Dougherty & Lotufo, 2003) and quadtree decomposition (Gonzalez & Woods, 2007). These techniques however are well-known to produce over-segmentation, *i.e.*, they find more objects than it is needed. Figure 2 presents the segmentation of the image presented in Figure 1-top using watershed (Figure 2-top) and quadtree decomposition (Figure 2-bottom).

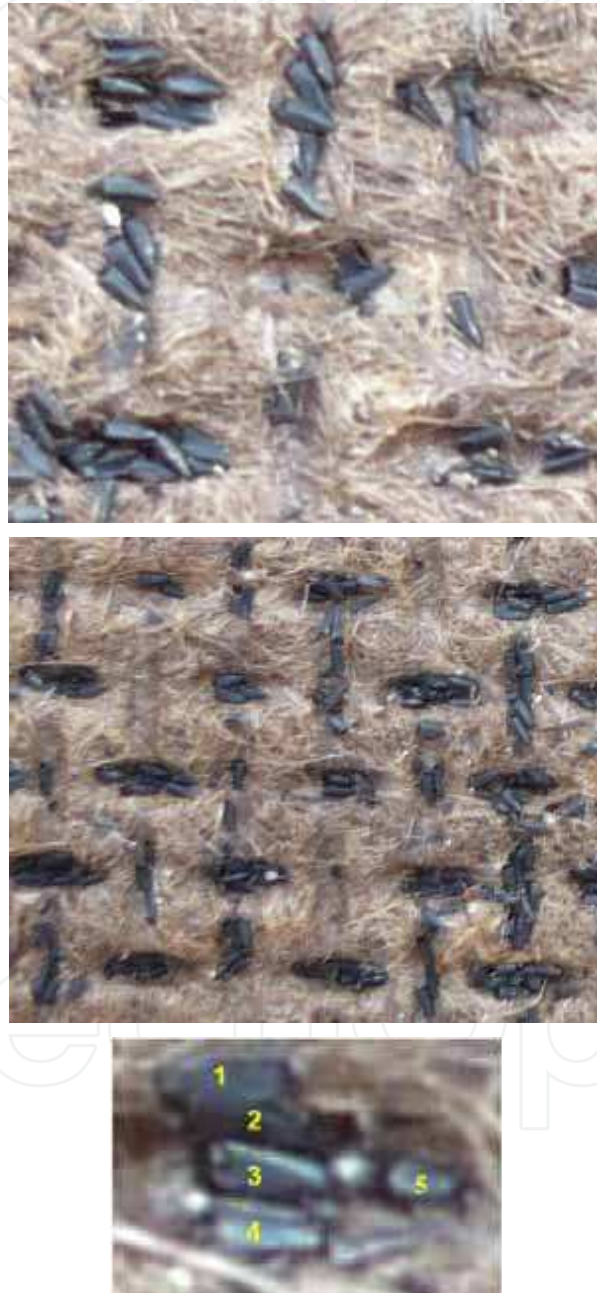


Fig. 1. Samples of an ovitrap with: (top) 34 eggs and (center) 111 eggs (bottom). A zooming into a group of five eggs (labeled in yellow).



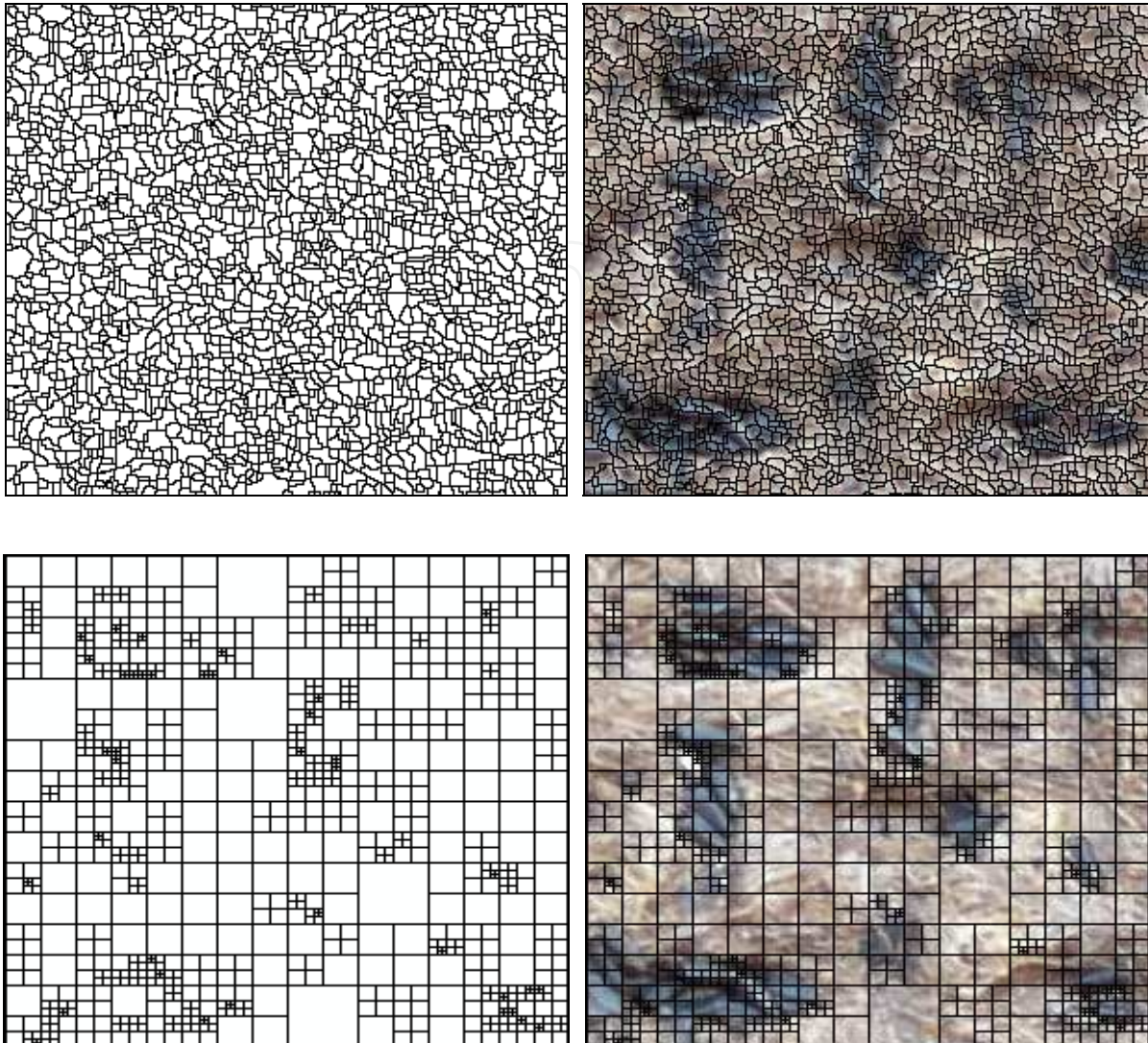


Fig. 2. Segmentation results using (top) watershed and (bottom) quadtree decomposition.

Other texture based methods groups areas that are considered similar based on some feature as texture analysis. The image is broken into  $k$  windows. Each window  $w_r$ ,  $1 \leq r \leq k$ , is compared with the others. If the windows  $w_i$  and  $w_j$  satisfies some criteria, they are considered the same texture. In this case, the criteria is a fidelity index. This technique was previously presented in (Mello & Mascaro, 2006) for mammographic image analysis and it uses a fidelity index (Wang & Bovik, 2002) to merge each windows. Figure 3 presents the 36 different areas found in the sample image of Figure 1-top based on texture features. Different colors are used to represent each different area; similar areas must appear in different regions of the image. The problem of over-segmentation can be noticed.

Two methods are proposed for the automatic counting. Each one of them is based on a different color space model. Next, we will detail both of them.

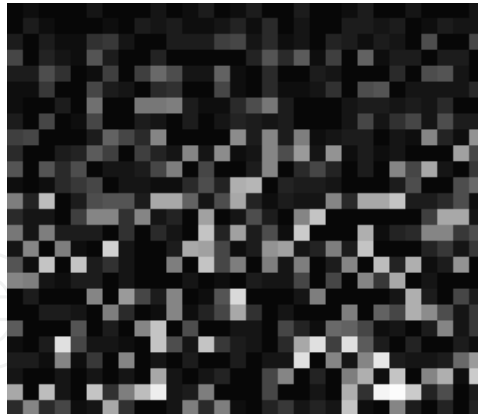


Fig. 3. Segmentation of image of Figure 1-top using a fidelity index to merge similar regions (each different color represents a different region).

### 3.1 First Method

Trying to achieve more difference between the eggs and the trap, the images are converted from RGB to HSL color model (*Hue, Saturation and Lightness*). One can see in Figure 4 the HSL components of the image shown in Figure 1-top.

From these three components, the hue is the one that contains information about the color tone. For example a hue value of 240 is related to several blue tones. It is evaluated as (Ballard & Brown, 1982):

$$hue = \cos^{-1} \left( \frac{(((r - g) + (r - b)) / 2)}{\sqrt{(r - g)^2 + (r - g)(g - b)}} \right) \quad (1)$$

where  $r$ ,  $g$  and  $b$  are the values of the red, green and blue components for a given color. As can be seen in Figure 4-left, the hue does not retain information about most part of the ovitrap itself.

The hue image is then binarized using Huang thresholding algorithm (Huang & Wang, 1995). Other thresholding algorithms (Sezgin & Sankur, 2004) were tested but Huang's and Li-Lee's algorithms produced the best results.

Huang algorithm uses a function  $E(t)$  that is applied to all possible threshold values ( $t$ ). When the smallest value of the function is found the corresponding value of  $t$  is chosen as the threshold. The function  $E(t)$  is defined as:

$$E(t) = 1 / (m.n) \sum Hf(Ux(g)).h(g) \quad (2)$$

where:

$g$  = gray level

$h(g)$  = histogram level for a given gray level

$n$  = number of lines in the picture

$m$  = number of columns in the picture

$Ux$  and  $Hf$  are two values found from a pre-defined set of equations.

The Li-Lee method is an entropy-based technique. It finds the threshold value by minimizing the cross entropy between the image and its segmented version.

To understand this algorithm, we must understand the concept of maximum entropy and from it, minimum cross entropy. The principal of maximum entropy allows us to choose the solution that outputs greatest entropy. It has been shown through experiments that distributions that have greater entropy have higher multiplicity, therefore being more likely to be observed.

Cross entropy measures the theoretical distance between two distributions:  $P=\{p_1, p_2, p_3, \dots, p_n\}$  and  $Q=\{q_1, q_2, q_3, \dots, q_n\}$  by:

$$D(Q, P) = \sum q_k \log_2 q_k / p_k \quad (3)$$

The minimum cross entropy method can be seen as an extension of the maximum cross entropy method, assigning initial estimated values for all  $p_i$  when no information is available.

This algorithm functions in a way that it is considered as a reconstruction process of the image distribution. The segmented image  $g(x,y)$  will be constructed in the following manner:

$$g(x, y) = \begin{cases} u1, & f(x, y) < t \\ u2 & f(x, y) \geq t \end{cases} \quad (4)$$

The segmented image  $g(x,y)$  will be solely determined by the function  $f(x,y)$  which has two unknown variables,  $\mu_1$ ,  $\mu_2$ , and  $t$ . A criteria function must be used to determine the best values for these variables so that they best satisfy  $f$ . The criteria function in this method is the cross entropy. It will be associated with the functions shown above, finding the threshold  $t$  and the final image  $g(x,y)$ .

We opted by Huang's algorithm because of the lower processing time. Figure 5-left shows the bi-level version of the hue image of Figure 4-left. There are still several parts considered objects in the image as they are converted into white.

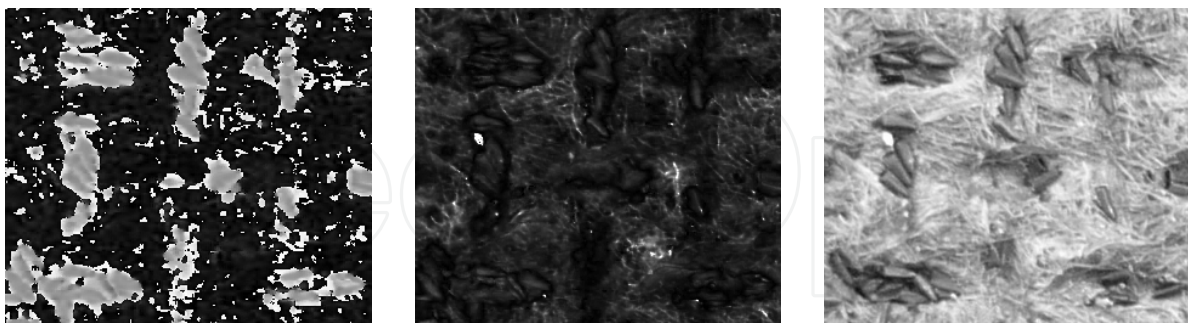


Fig. 4. HSL components of the image in Figure 1-top: (left) Hue components; (center) Saturation and (right) Lightness.

With the bi-level image, a connected components algorithm is applied to label the connected regions of the image (Shapiro & Stockman, 2001). This algorithm puts a different label at each connected white area of the image. With this labeling, it is possible to evaluate each connected area. Small areas can be deleted as it could not contain an egg. Our experiments defined that every area with less than 100 pixels should be deleted. This can be seen in

Figure 5 - right where it is presented the image of Figure 5 - left after the reduction of its white areas.



Fig. 5. (left) Hue image after binarized by Huang's thresholding algorithm and (right) bi-level hue image after elimination of small connected areas.

After this, the image is filtered using the morphological operation of closing (Parker, 1997). For this purpose, a structural element is defined in the form of an egg. As the eggs are disposed in different positions along the ovitrap, it was selected a sample egg with average size and with a small inclination angle. To avoid the loss of small eggs in the counting process, the image used as structural element has its original size reduced from  $18 \times 30$  pixels to  $8 \times 13$  pixels. Figure 6 presents the original image (left-top) and the final structural element (left-bottom).

The result of the closing operation applied to the image presented in Figure 5-right is shown in Figure 6-right. The areas with eggs are now more delimited.



Fig. 6. Egg identification. (a) Average egg that was used to define (b) the structural element. (c) Image of Figure 5-right after application of the closing operator with the structural element of Figure 6-left-bottom.

For the final stage, we considered that an egg occupies an area of 170 pixels. So, the number of eggs is the total amount of white pixels divided by this average area. In this case, the method registered an amount of 33 eggs against the correct value of 34 eggs that the image contains.

### 3.2 Second Method

The second approach used in this work is based on converting RGB sub-images to YIQ ones and, finally, segmenting band I and counting mosquitoes eggs using a standard labeling



algorithm (Gomes, Velho & Frery, 2008). YIQ color base transformation is given as follows (Ballard & Brown, 1982):

$$\begin{bmatrix} Y \\ I \\ Q \end{bmatrix} = \begin{bmatrix} 0.299 & 0.587 & 0.114 \\ 0.596 & -0.275 & -0.321 \\ 0.212 & -0.523 & 0.311 \end{bmatrix} \begin{bmatrix} R \\ G \\ B \end{bmatrix}. \quad (5)$$

The segmentation of band I can be performed in two ways: 1) using limiarization with a fixed threshold of 200; 2) binarization using k-means clustering method (Haykin, 1998), with 3 inputs, 4 classes, learning rate of 0.1 and a maximum of 200 iterations. To perform eggs counting, it is considered an average-sized mosquito egg of 220 pixels. Such a difference of size (220 pixels against about 250 of the first method) is due to different segmentation methods and the absence of the application of mathematical morphology-based filters in this method, once there is no structural element.

Figure 7-top-left presents an RGB color sub-image of an ovitrap, where Figure 7-top-right shows the RGB composition of its YIQ version. Figure 7-bottom-left presents segmentation results using the fixed threshold approach, whereas Figure 7-bottom-right shows the k-means based method. It can be seen in Figure 8 and Figure 9, respectively, the gray level version and histogram of band I of the image presented in Figure 7-top-right. In this example, the proposed algorithm counted 34 eggs, exactly the same number of eggs got by the visual non-automatic counting process.

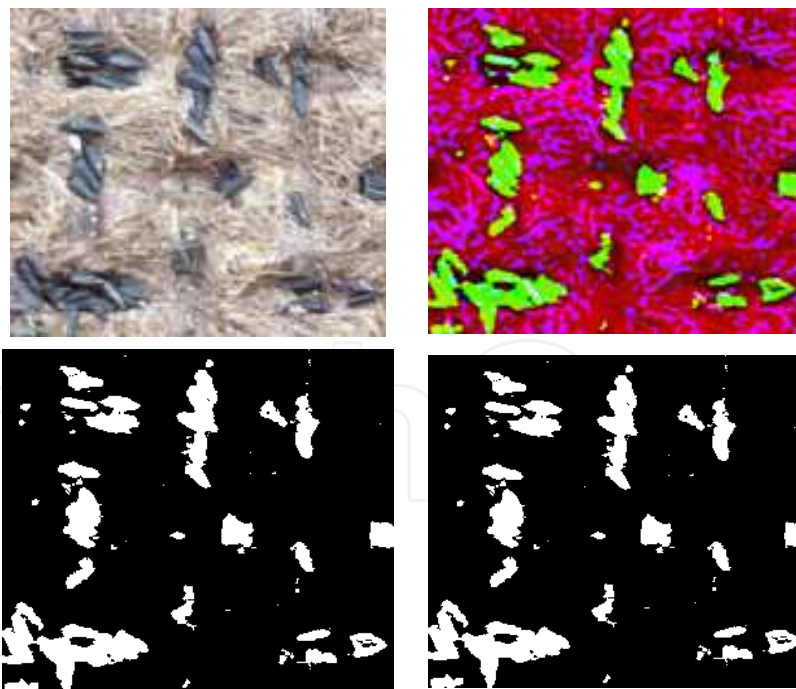


Fig. 7. (top-left) RGB color sub-image of an ovitrap, (top-right) RGB composition of its YIQ representation, and (bottom-left) segmentation of its band I with a fixed limiar of 200 and (bottom-right) a k-means classifier.

4. Discussion and Results

In Table 1, it is presented the results of the two methods applied to another six samples, including an image without eggs. The image labeled as ‘3’ in this Table is the image previously presented in Figure 1-right with 111 eggs.

Figure 8 presents bands of YIQ and Figure 9 shows the histogram of I band. It can be seen that the histogram presents two maximum: one near of zero and other one near of 255 (mosquito eggs).

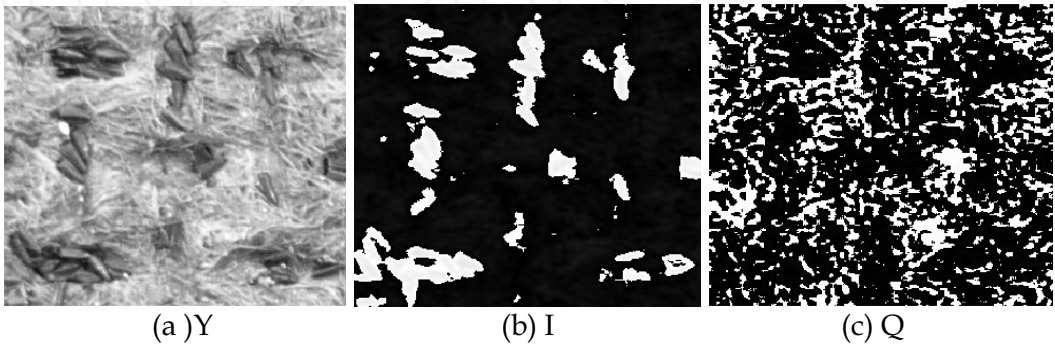


Fig. 8. YIQ model of sub-image on Figure 7-top-left.

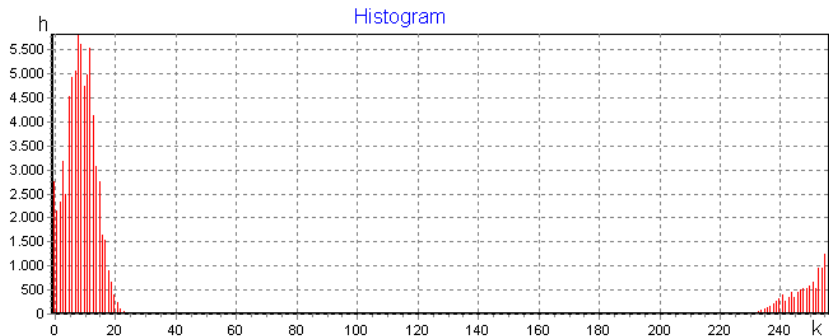


Fig. 9. Histogram of Band I of the RGB sub-image presented in Figure 7-top-left.

Image	Correct Amount of Eggs	Estimated Amount of Eggs by the Proposed Algorithms		
		Method 1	Method 2 Fixed Threshold	Method 2 k-Means
1	22	25	29	20
2	8	10	10	6
3	111	111	113	107
4	30	26	32	28
5	19	19	21	19
6	0	0	0	0

Table 1. Counting results using the proposed methods

The first method reached a maximum error of 25% in the second image where there is a difference of two eggs. But in average the error was about 6.66% which is acceptable in comparison with a non-automatic method.

The version of the second method with segmentation based on unsupervised classification of the YIQ image furnish better results than the other, based on binarization of band I with a fixed threshold of 200. This result is due to the use of all 3 bands of YIQ image, despite the fact that band I is perfectly feasible to be used as input to segmentation algorithm, as can be observed in Figure 7 and Figure 8. The second method using a fixed threshold value for binarization achieved an average error rate of 7.33%, while the use of k-Means produced an average error of 7.84%.

Both methods achieved very satisfactory results. Other color spaces must be tried in search for better responses.

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The field of biomedical engineering has expanded markedly in the past ten years. This growth is supported by advances in biological science, which have created new opportunities for development of tools for diagnosis and therapy for human disease. The discipline focuses both on development of new biomaterials, analytical methodologies and on the application of concepts drawn from engineering, computing, mathematics, chemical and physical sciences to advance biomedical knowledge while improving the effectiveness and delivery of clinical medicine. Biomedical engineering now encompasses a range of fields of specialization including bioinstrumentation, bioimaging, biomechanics, biomaterials, and biomolecular engineering. Biomedical engineering covers recent advances in the growing field of biomedical technology, instrumentation, and administration. Contributions focus on theoretical and practical problems associated with the development of medical technology; the introduction of new engineering methods into public health; hospitals and patient care; the improvement of diagnosis and therapy; and biomedical information storage and retrieval. The book is directed at engineering students in their final year of undergraduate studies or in their graduate studies. Most undergraduate students majoring in biomedical engineering are faced with a decision, early in their program of study, regarding the field in which they would like to specialize. Each chosen specialty has a specific set of course requirements and is supplemented by wise selection of elective and supporting coursework. Also, many young students of biomedical engineering use independent research projects as a source of inspiration and preparation but have difficulty identifying research areas that are right for them. Therefore, a second goal of this book is to link knowledge of basic science and engineering to fields of specialization and current research. The editor would like to thank the authors, who have committed so much effort to the publication of this work.

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